METHODS FOR ENCAPSULATING PLASMIDS IN LIPID BILAYERS

This application is a continuation of and claims the benefit of U.S. application Ser. No. 08/484,282, filed Jun. 7, 1995, now U.S. Pat. No. 5,981,501, the disclosure of which is incorporated by reference.

FIELD OF THE INVENTION

This invention relates to formulations for therapeutic nucleic acid delivery and methods for their preparation, and in particular to lipid encapsulated plasmids or antisense constructs. The invention provides a circulation-stable, characterizable delivery vehicle for the introduction of plasmids or antisense compounds into cells. These vehicles are safe, stable, and practical for clinical use.

BACKGROUND OF THE INVENTION

Gene therapy is an area of current interest which involves the introduction of genetic material into a cell to facilitate expression of a deficient protein. There are currently five major methods by which this is accomplished, namely: (i) calcium phosphate precipitation, (ii) DEAE-dextran complexes, (iii) electroporation, (iv) cationic lipid complexes and (v) reconstituted viruses or virosomes (see Chang, et al., *Focus* 10:88 (1988)). Cationic lipid complexes are presently the most effective generally used means of effecting transfection.

A number of different formulations incorporating cationic lipids are commercially available, namely (i) LIPOFECTIN® (which uses 1,2-dioleyloxy-3-(N,N,N-trimethylamino) propane chloride, or DOTMA, see Eppstein, et al., U.S. Pat. No. 4,897,355); LIPOFECTAMINE® (which uses DOSPA, see Hawley-Nelson, et al., *Focus* 15(3):73 (1993)); and LIPOFECT ACE® (which uses N,N-distearyl-N,N-dimethylammonium bromide, or DDAB, see Rose, U.S. Pat. No. 5,279,833). Others have reported alternative cationic lipids that work in essentially the same manner but with different efficiencies, for example 1,2-dioleoyloxy-3-(N,N,N-trimethylamino)propane chloride, or DOTAP, see Stomatatos, et al., *Biochemistry* 27:3917–3925 (1988)); glycerol based lipids (see Leventis, et al., *Biochem. Biophys. Acta* 1023:124 (1990); lipopolyamines (see, Behr, et al., U.S. Pat. No. 5,171,678) and cholesterol based lipids (see Epand, et al., WO 93/05162, and U.S. Pat. No. 5,283,185).

Others have noted that DOTMA and related compounds are significantly more active in transfection assays than their saturated analogues (see, Felgner, et al., WO91/16024). However, both DOTMA and DOSPA based formulations, despite being the most efficient of the cationic lipids in effecting transfection, are prohibitively expensive. DDAB on the other hand is inexpensive and readily available from chemical suppliers but is less effective than DOTMA in most cell lines. Another disadvantage of the current lipid systems is that they are not appropriate for intravenous injection.

An examination of the relationship between the chemical structure of the carrier vehicle and its efficiency of transfection has indicated that the characteristics which provide for effective transfection would make a carrier unstable in circulation (see, Ballas, et al., *Biochim. Biophys. Acta* 939:8–18 (1988)). Additionally, degradation either outside or inside the target cell remains a problem (see, Duzghines, *Subcellular Biochemistry* 11:195–286 (1985)). Others who have attempted to encapsulate DNA (Szoka et al., *Ann. Rev. Biophys. Bioeng.* 9:467 (1980); and Deamer, U.S. Pat. No. 4,515,736) made no efforts to ensure a safe, injectable

formulation, or arrived at inefficient loading (Legendre, *Pharm. Res.* 9:1235–1242 (1992)).

Ideally, a delivery vehicle for a nucleic acid or plasmid will have the following characteristics: a) small enough and long lived enough to distribute from local injection sites when given intravenously, b) capable of carrying a large amount of DNA per particle to enable transfection of all sizes of genes and reduce the volume of injection, c) homogenous, d) reproducible, e) protective of DNA from extracellular degradation and f) capable of transfecting target cells in such a way that the DNA is not digested intracellularly.

The present invention provides such compositions and methods for their preparation and use.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides methods for the preparation of serum-stable plasmid-lipid particles. In one group of these methods, a plasmid is combined with cationic lipids in a detergent solution to provide a coated plasmid-lipid complex. The complex is then contacted with non-cationic lipids to provide a solution of detergent, a plasmid-lipid complex and non-cationic lipids, and the detergent is then removed to provide a solution of serum-stable plasmid-lipid particles, in which the plasmid is encapsulated in a lipid bilayer. The particles, thus formed, have a size of about 50–150 nm.

In a related group of methods the serum-stable plasmid-lipid particles are formed by preparing a mixture of cationic lipids and non-cationic lipids in an organic solvent; contacting an aqueous solution of plasmid with the mixture of cationic and non-cationic lipids to provide a clear single phase; and removing the organic solvent to provide a suspension of plasmid-lipid particles, in which the plasmid is encapsulated in a lipid bilayer, and the particles are stable in serum and have a size of about 50–150 nm.

In another aspect, the present invention provides plasmid-lipid particles prepared by the above methods.

In yet another aspect, the present invention provides methods of transfecting cells using these plasmid-lipid particles.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** illustrates a liposome-mediated transfection using "sandwich-type" complexes of DNA.

FIG. **2** illustrates an aggregation and precipitation which commonly occurs during the entrapment of large nucleic acids in lipid complexes.

FIG. **3** provides a schematic representation of the preparation of plasmid-lipid particles using the methods of the present invention.

FIG. **4** illustrates the recovery of ^{3}H-DNA from encapsulated particles following the reverse-phase preparation of the particles and extrusion through a 400 nm filter and a 200 nm filter. Lipid composition is POPC:DODAC:PEG-Cer (C_{20}) in proportions as shown in Table 1.

FIG. **5** illustrates the recovery of ^{3}H-DNA from particles prepared using a reverse-phase procedure. The particles were extruded through a 200 nm filter and eluted on a DEAE Sepharose CL-6B anion exchange column. The percent recovery reported is based on the amount recovered after filtration. Lipid composition is as in FIG. **4**.

FIG. **6** illustrates the recovery of ^{14}C-lipid from encapsulated particles following the reverse-phase preparation of